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Exposure to IFNα Leads to Generation of IL-10-producing Type 1 regulatory cells

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Background

Chronic immune activation is a hallmark of HIV-1 infection and one of the strongest predictors of disease progression. A dysregulation in cytokine production as well as elevated serum levels of interferon alpha (IFNα) and Interleukin-10 (IL-10) are associated with immune hyperactivation. Plasmacytoid dendritic cells (pDCs) are the main producers of IFNα progressively decrease in peripheral blood (pB) at later stages of disease and relocate to lymphoid tissues where they produce IFNα (Fig. 1). Recently, coexpression of CD49b and LAG-3 was shown to identify Type 1 regulatory (Tr1) cells (Gagliani N et al.) , which secrete large amounts of the immune-suppressive cytokine IL-10. In this study we compared different patient groups regarding Tr1 frequency and IL-10 expression and additionally aimed at assessing whether IFNα production during HIV infection directly causes immune activation and increased expression of Tr1 cells.

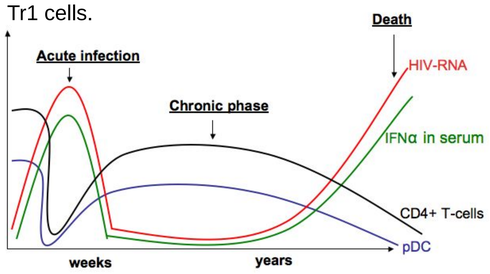


***Figure 1: Disease progression is associated with increase of IFNα in peripheral blood**. HIV-RNA, CD4+ T cells, pDC and IFN-α-levels during the natural disease progression of HIV-infection*
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Methods

PDCs (BDCA2+CD123+) and Tr1 (CD3+CD4+CD49b+LAG-3+) cells in peripheral blood of seronegative controls (*n*=10) and HIV-1$^+$ subjects (viremic: *n*=12; long-term nonprogressors (LTNPs): *n*=6) were quantified by flow cytometry. Spontaneous IFNα and IL-10 expressions were assessed by cytokine secretion assays. Data (expressed as mean and standard error of the mean, SEM) were analyzed using Mann Whitney tests. In an *in vitro* model of primary antigen-driven immune response, CD4+ T cells from uninfected donors were activated and expanded for 7 days with monocyte-derived dendritic cells (MDDC) in the presence (or absence) of 10 ng/ml IFN-α. We used cytokine expression to profile CD4+ T cell subsets (IFN-γ and IL-2 for Th1; IL-4 for Th2; IL-10 for Tr1; IL-17 for Th17). Frequency of Tr1 cells was measured 24 h after treatment with 10 ng/ml IFNα.

Table 1: Clinical parameters of the different study groups. Values for analytical results are presented as "median (range)".

	Control	HIV	LTNP
gender	7 ♀, 3 ♂	3 ♀, 9 ♂	2 ♀, 4 ♂
age (years)	37 (26-57)	46.5 (30-65)	47 (29-53)
CD4+ T cells (cells/µl)	n.a.	275 (20-720)	745 (460-820)
viral load (copies/ml)	n.a.	62,767 (892-778,000)	170 (<40-411)
CDC category	n.a.	A1 (n=2), A2 (n=3), A3 (n=2), B2 (n=2), B3 (n=1), C3 (n=2)	A1 (n=1), A2 (n=5)

Results

We found higher frequency of circulating Tr1 cells in viremic HIV-1$^+$ patients (1.2% ± 0.5) vs. LTNPs (0.5% ± 0.2; p<0.05) and vs. controls (0.8% ± 0.3; p=0.006, Fig. 2 A). IL-10 expression (geometric mean fluorescence intensity, GMFI) and IL-10 expression on a per Tr1 cell basis was not significantly different among the groups (p>0.05. Fig. 2 B, C). In contrast, lower frequency of circulating pDC was observed in viremic (0.2% ± 0.02) vs. elite controllers (0.5% ± 0.07; p<0.01) vs. controls (0.8% ± 0.08; p<0.001, Fig. 2 D). Elevated IFNα secretion (GMFI) was found in viremic patients (12.4 ± 1.28) vs. LTNPs (4.4 ± 1.2; p=0.02) and controls (Mean 8.9 ± 2.9; p=0.012, data not shown).

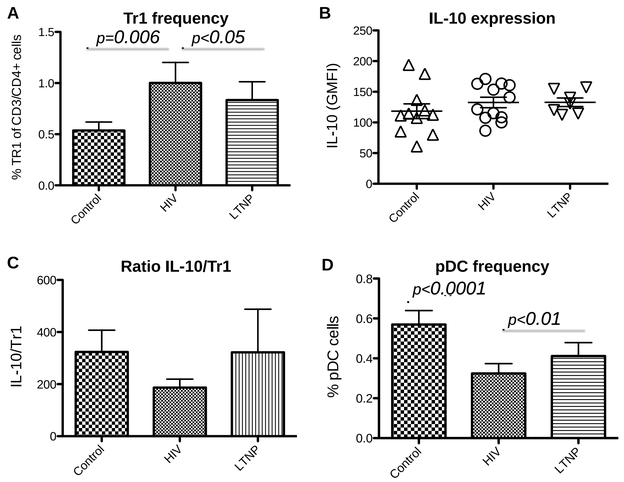


***Figure 2: Comparison of pDC and Tr1 frequency as well as cytokine expression in different study groups**. For cell frequency analysis, PBMCs were stimulated with anti-CD3/CD28 antibodies (Tr1) or CpG (pDC) and subsequently analyzed by flow cytometry. Cytokine expression was detected by secretion assay analyis. Expression levels are shown as Geometric Mean Fluorescence Intensity (GMFI) and all values for analytical results are presented as 'geometric mean'. (A) Tr1 population was identified by staining with anti-CD49b and anti–LAG-3 antibodies. (B) Flow cytometry plot showing expression of IL-10 by Tr1 cells. (C) Ratio of IL-10 cytokine expression to Tr1 population. (D) pDC population was identified by staining with anti-BDCA2 and anti–CD123 antibodies.*

Second, CD4+ T cells activated and expanded *in vitro* in the presence of IFNα expressed lower levels of Th1, Th2 and Th17 cytokines (IFN-γ and IL-2 for Th1; IL-4 for Th2; IL-10 for Tr1; IL-17 for Th17, data not shown) and higher levels of the Tr1 cytokine IL-10 (Fig. 3 A). Finally, incubation of isolated naive CD4+ T-cells with IFNα resulted in an increased frequency of Tr1 cells (Fig. 3 B).

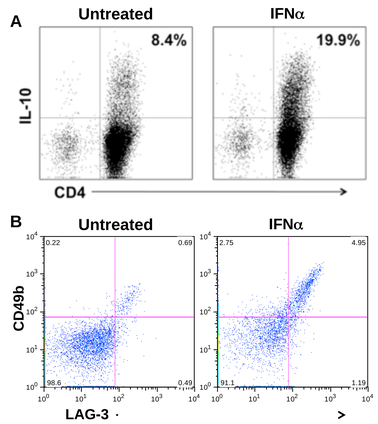


***Figure 3: Upregulation of IL-10 and Tr1 cells by IFNα.** IL-10 expression by CD4+ T cells (A) and Tr1 frequency within the CD4+ T cell population (B) from a representative HIV negative donor after 24 hours of stimulation with anti-CD3/CD28 antibodies in the absence (left) or presence (right) of IFN-α.*

Conclusions

This is the first study reporting that HIV patients show an elevated frequency of Tr1 cells. As expected, the pDC frequency is reduced in HIV patients, as these cells migrate from pB to lymphoid tissue.
Our data suggests that over-expression of IFNα during HIV-1 infection directly drives the generation of Tr1 cells as well as the immune suppressive cytokine IL-10. In ongoing studies we asses the question whether there is a link between IFNα and IL-10 production via Tr1 cells.

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